AD NUMBER

AD468162

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TECHNICAL MANUSCRIPT 234

AUXIN STIMULATION OF ETHYLENE EVOLUTION

Frederick B. Abeles

JULY 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
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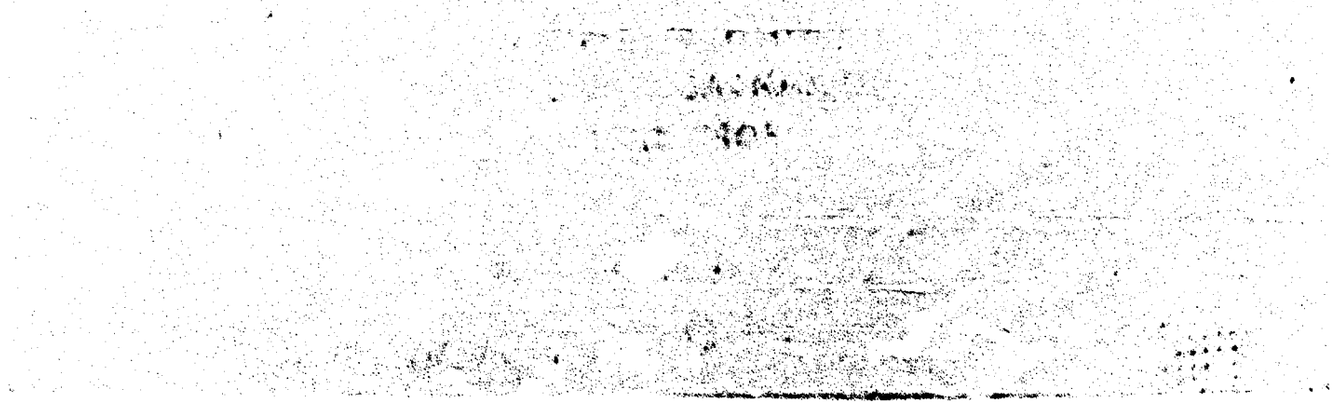

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 234

AUXIN STIMULATION OF ETHYLENE EVOLUTION

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Crops Division
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1L013001A91A

July 1965

ACKNOWLEDGMENTS

The author acknowledges the aid of Sp-4 A. Hartman, Lt. R. Holm, and Lt. T. Weymouth during the course of these experiments. We also wish to thank Merck, Sharp, and Dohme Research Laboratories of Merck & Co. for the gift of actinomycin D.

ABSTRACT

The stimulation of ethylene production by auxin was inhibited by actinomycin D and other inhibitors of protein synthesis. It is concluded that the mechanism of auxin action on the enhancement of ethylene production is the formation of enzymes involved in ethylene biogenesis.

I. INTRODUCTION

The stimulation of ethylene production is one of the effects of auxin on plants. Zimmerman and Wilcoxon[1] first noticed this phenomenon when tomato plants treated with indoleacetic acid (IAA) in lanolin produced a gas that caused an epinastic response in ethylene-sensitive plants. Later, Morgan and Hall[2] presented evidence that the gas produced is ethylene by showing it was possible to trap the gas as a mercuric perchlorate complex. Abeles and Rubinstein[3] found that auxin would stimulate ethylene production from roots, stems, and leaves of nine different genera and that endogenous levels of auxin also regulated the production of ethylene from vegetative tissues.

Auxin has also been shown to stimulate protein synthesis through a regulation of nucleic acid metabolism. Key and Shannon[4] found that 2,4-dichlorophenoxyacetic acid (2,4-D) and IAA would stimulate the uptake of C^{14} adenosine triphosphate into the ribonucleic acid (RNA) of the ribosomal function of soybean hypocotyls after an initial lag period of about 3 hours and that actinomycin D inhibited the incorporation of the nucleotide by 85 to 90%. Key[5] found that 2,4-D would also stimulate incorporation of C^{14} leucine into soybean hypocotyl protein and that actinomycin D, 8-azaguanine, and puromycin would act as inhibitors. Fang and Chang Y.[6] also reported that after an initial lag, 2,4-D and IAA stimulated the incorporation of glycine into water-soluble proteins.

This paper describes experiments that examine the possibility that auxin-enhanced ethylene production is mediated through the production of specific enzymes required for the enhanced synthesis of ethylene.

II. MATERIALS AND METHODS

Ethylene was measured by gas chromatography as described earlier.[3] Seven-day-old etiolated beans (*Phaseolus vulgaris* L. var. Red Kidney), seven-day-old sunflower (*Helianthus annuus* L. var. Russian mammoth) and four-day-old corn (*Zea mays* L. var. Burpee's Barbecue Hybrid) were grown on moist vermiculite in the dark at 25 C and a relative humidity of about 70 to 80%. A red light was turned on for three hours during the third night of growth of corn seedlings to inhibit mesocotyl elongation. Bean hypocotyl tissue consisted of 20 one-mm sections of tissue cut

immediately below the crook, sunflower hypocotyl tissue consisted of two hypocotyls cut below the cotyledonary node into 14 one-mm sections, and corn tissue consisted of four coleoptile tips cut into 14 one-mm sections. These tissues were placed into 5-ml syringes, sealed with rubber vaccine caps, in 1 ml of liquid phase and a gas phase of 3 ml. The syringes were shaken 80 times per minute with an amplitude of 2 cm at 25 C, then 2 ml of the gas phase was withdrawn for ethylene measurements. Since, as reported earlier[3] a two-hour lag period occurs between addition of auxin and stimulation of ethylene production, syringes were vented after two hours, sealed, and ethylene was measured after two additional hours of shaking. Each experiment was conducted four times and data represent the mean plus or minus the standard deviation.

III. RESULTS

The hypothesis that the stimulation of ethylene evolution is mediated through an increased synthesis of enzymes involved in ethylene formation was tested by adding actinomycin D, puromycin, p-flurophenylalanine, and 2-thiouracil. These compounds are known to inhibit protein synthesis at different points of the synthetic pathway. Actinomycin D is known to block RNA synthesis that is dependent upon deoxyribose nucleic acid (DNA); puromycin inhibits the coupling of amino acids by acting as an analogue of transfer RNA; incorporation of p-fluorophenylalanine results in the formation of abnormal proteins; and 2-thiouracil causes the formation of nonsense RNA. The results of the effect of these compounds on 2,4-D stimulated ethylene evolution from bean hypocotyls are shown in Table 1. The results of additional experiments with other growth regulators and other species of plants are summarized in Figure 1.

The data indicate that there is little or no effect of these inhibitors on endogenous rates of ethylene production. All, however, inhibited auxin-stimulated ethylene evolution. The most effective inhibitors were actinomycin D and puromycin, which is the expected result considering their specificity of action at key points in protein synthesis. 2-Thiouracil and p-fluorophenylalanine are only partially effective as inhibitors. These results may be due either to penetration problems or to the presence of large pools of the normal analogues inside the cell.

TABLE 1. EFFECT OF PROTEIN SYNTHESIS INHIBITORS ON THE STIMULATION OF ETHYLENE EVOLUTION BY 2,4-D[a]/

Inhibitor	Nanoliters Ethylene Evolved ± Standard Deviation				
	Control	Inhibitor	2,4-D	2,4-D + Inhibitor	Inhibition, %
Actinomycin D	1.2±0.3	2.0±1.5	10.5±2.2	1.7±0.3	84
Puromycin	1.5±0.5	1.8±0.5	11.5±1.0	7.3±2.2	36
2-Thiouracil	2.2±0.7	2.0±0.5	9.0±1.0	6.7±1.0	31
p-Fluorophenylalanine	2.0±0.5	3.0±1.0	8.2±1.5	6.8±1.7	17

a. Twenty 1-mm sections of bean hypocotyls were placed in 5-ml syringes (1 ml liquid volume, 3 ml gas volume) and shaken 80 times per minute with an amplitude of 2 cm at 25 C. 2,4-D, 0.1 µmole; actinomycin D, 10 µg; puromycin, 0.2 µmole; 2-thiouracil, 10 µmoles; p-fluorophenylalanine, 10 µmoles. Ethylene evolution was measured between 2 and 4 hours.

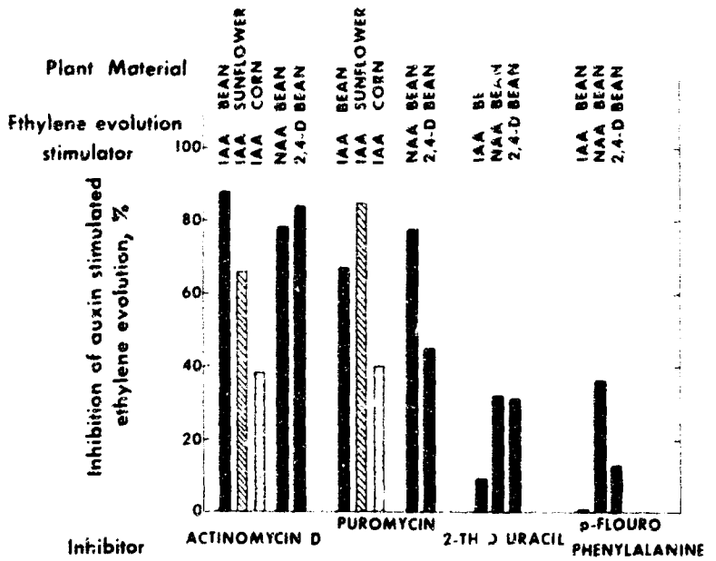

Figure 1. Effect of Protein Synthesis Inhibitors on the Stimulation of Ethylene Production by Growth Regulators. Each syringe contained one bean hypocotyl, 20 one-mm sections; two sunflower hypocotyls, 14 one-mm sections; or four corn coleoptiles, 14 one-mm sections. These sections were placed in 1 ml (3 ml gas volume) solutions of IAA, NAA, 2,4-D, 0.1 µmole; actinomycin D, 10 µg; puromycin, 0.2 µmole for beans, 0.1 µmole for corn and sunflower; 2-thiouracil, 10 µmoles; p-fluorophenylalanine, 10 µmoles. Temperature 25 C.

Hall and Lane[7] and Jackson[8] have shown that defoliants are capable of stimulating ethylene production from leaves. More recently we have shown[9] that endothal (3,6-endoxohexahydrophthalic acid) and KI are capable of stimulating ethylene evolution from excised bean abscission zones. However, addition of these compounds to bean hypocotyls had no effect on ethylene evolution for the first 6 hours of incubation. It is possible that the mode of action of these compounds is through yet another mechanism as compared with the auxins, which apparently act primarily on the protein synthesizing systems.

IV. DISCUSSION

There is a good deal of satisfactory evidence[5,6,10] that auxins are capable of stimulating the formation of new proteins and there is also adequate documentation[3] that auxin is capable of stimulating the production of ethylene. Through the use of protein synthesis inhibitors we have shown that the action of auxin on increased ethylene production is probably mediated through the enhanced production of those enzymes involved in ethylene biogenesis, although at this time the exact biochemical pathway of ethylene synthesis remains unknown. In addition, many workers[4,6,10] have shown that there is a lag period of about 2 hours between the addition of auxin and the enhanced synthesis of proteins. This observation also agrees with the lag period observed between application of auxin and an enhancement of ethylene production.

The enzymes involved in ethylene biogenesis are probably only a small part of those whose production is stimulated by auxin. Baker and Ray[11] and Ray and Baker[12] have shown that IAA stimulates the incorporation of glucose into the cell walls of oat coleoptiles whose growth was inhibited by Ca^{++} ions. This enhancement is not a direct incorporation of glucose into the cellulose but rather a conversion of glucose into other carbohydrates such as glucans, arabinans, and pectic acid. These conversions are mediated by enzymes that apparently increase in amount by the action of IAA. Their observation of a lag period of about an hour between addition of IAA and stimulation of the incorporation and conversion of glucose is the anticipated result if an hypothesis of enhanced protein synthesis is proposed.

Hormonal control of protein synthesis is not limited to auxins. Paleg[13] reported that gibberellic acid stimulated the production of alpha-amylase from barley endosperm. Later, Varner[14] reported that under appropriate conditions it was possible to prevent gibberellin-dependent de novo synthesis of alpha-amylase by nucleic acid analogues and actinomycin D.

The observation that auxin action is in part mediated through control of protein biosynthesis suggests a means by which a single molecule can influence a variety of different metabolic pathways and how the effectiveness of a small amount of hormone can control major biochemical pathways. However, the method by which plant hormones interact with the nucleus to regulate nucleic acid metabolism is as yet unknown.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)
U.S. Army Biological Laboratories
Fort Detrick, Frederick, Maryland, 21701

2a REPORT SECURITY CLASSIFICATION
Unclassified

2b GROUP

3 REPORT TITLE
AUXIN STIMULATION OF ETHYLENE EVOLUTION

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)
Abeles, Frederick B.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
July 1965	14 pages	14

8a CONTRACT OR GRANT NO.	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO 1L013001A91A	Technical Manuscript 234
c	9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d	

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11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland, 21701

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DD FORM 1473

Unclassified
Security Classification